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Effects of dietary protein level and source on the growth and survival of two genetic lines of specific-pathogen-free Pacific white shrimp, *Penaeus vannamei*

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ABSTRACT

An eight-week trial compared the performances of two genetic lines of *Penaeus vannamei*, T-line and G-line, fed with different levels and sources of dietary protein. Both lines were originated from the same founder populations but had gone through genetic selection targeting different breeding goals for more than four generations. T-line was bred for fast growth and Taura syndrome viral disease resistance, and G-line was bred for fast growth and production yield under super-high density. Five dietary treatments were employed: two commercial diets and three semipurified diets (A, B, and C). The semipurified diets were formulated at similar levels of digestible energy. Diet A contained 35% crude protein (CP), from marine meals (fish, 15%, and squid, 15%). Diet C contained 35% CP from a reduced marine protein level from fish (11.5%) and squid meals (11.5%). Diet B contained only 20% CP but had the same level of marine protein as diet C. The two commercial shrimp feeds, containing 35% and 40% protein, served as reference diets. Three replicate tanks per dietary treatment were used. Each tank was stocked with 50 tagged shrimp (25 each of T-line and G-line) and 100 untagged shrimp, with average initial weight (IWT) of 10.4 g, at a density of 107/m^2.
Diet significantly affected shrimp growth ($p<0.0001$) but did not interact with shrimp line. Shrimp fed either diet A or diet C grew over 25% faster than those fed diet B or either of the two commercial diets.
Diets A and C yielded similar growth (0.30 to 0.33 g/day) and survival rates (83 to 93%) to a harvest size of over 29 g per shrimp, suggesting that the soy protein isolate that replaced part of the marine protein in diet C can serve as a good alternative protein source. Growth rates of the two shrimp lines were similar regardless of dietary protein levels or sources, but dietary plant protein level did interact with shrimp line when the measure was survival: T-line survived better than G-line on average when fed diet B, a nutritionally inferior diet containing 20% protein and no plant protein, but not when fed diet C, containing 35% protein, 15% of which was of plant origin (soybean). Microsatellite marker analyses quantified the genetic relationship of the two lines and confirmed that they were the most closely related of the lines derived from the same founding stock. This preliminary study provided information that will be useful for further studies of the relationship between dietary nutrition and genetics and for exploration of the potential of genetic selection for improving the efficiency of plant protein use in shrimp.

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1. Introduction

Penaeus vannamei Boone, indigenous to the Pacific coast from Mexico southwards to Peru, has become the dominant penaeid shrimp cultured worldwide and accounts for over 66% of shrimp aquaculture production, which totaled 3.49 million metric tons globally in 2009 (FAO, 2011). This species has several merits that make it more suitable for aquaculture than other penaeid species, such as relatively low dietary protein requirements, high density tolerance, and adaptability to wide ranges of several environmental parameters, such as salinity and temperature. Moreover, tremendous research efforts have led to the development of genetically improved and specific-pathogen-free (SPF) stock, contributing greatly to *P. vannamei*'s growing popularity for aquaculture production (Argue et al., 2002; Lightner et al., 2009; Moss et al., 2001, 2007, 2010; Rocha et al., 2010).

As the dominant penaeid species of present-day aquaculture, *P. vannamei* also faces great challenges in terms of long-term and more sustainable development. Among these challenges are to seek cost-effective alternative protein sources to replace fish meal in shrimp feed and to increase efficiency of protein use by the species. Previous work has focused mainly on the replacement of protein of marine origin and showed that replacing it with plant-based protein is feasible with proper nutritional adjustment (Amaya et al., 2007; Davis and Arnold, 2000; Samocha et al., 2004). Soybean protein is

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high on the list of possible alternative protein sources because of its low cost, consistent quality and supply, and constant composition (Amaya et al., 2007). As much as 75% of the dietary protein for *P. vannamei* can be replaced with soy protein concentrate (Forster et al., 2002), but if fishmeal and oil are eliminated from shrimp diets entirely, an alternate source of docosahexaenoic omega-3 fatty acids (required for optimal shrimp growth) must be included in the feed (Galitzine et al., 2009).

The selective genetic breeding of *P. vannamei* started in the early 1990s (Moss et al., 2001, 2010). Breeding goals have emphasized the selection for fast growth, density tolerance, and resistance to Taura syndrome virus (TSV) and other diseases, and have produced promising results (Moss et al., 2007, 2010, 2011). An average genetic gain in shrimp growth rate of over 10% per generation was observed (Argue et al., 2002; Rocha et al., 2007).

Whether the continuous genetic improvement in production traits, which might have changed the underlying biological or physiological processes, has altered the shrimp's nutritional requirements is unknown, and no published studies have addressed whether *P. vannamei* shows any genetic variability in the efficiency of use of plant protein.

We planned to conduct a series of studies to investigate the potential interaction between shrimp genetics and dietary nutrition for *P. vannamei* and to explore the possibilities for improving the efficiency of use of dietary plant protein by means of genetic selection. The work reported here was an initial step designed to determine whether selective breeding for different traits has altered *P. vannamei*'s two most important aspects of dietary protein requirement: protein level and protein source. The specific objectives of the present study were (1) to investigate the performance of two different shrimp lines, under conditions of high density, fed with semipurified diets containing different protein levels and different ratios of marine protein to plant protein and (2) to determine whether the experimental system and base diet formulation are adequate for further study of the interaction between shrimp genetics and nutrition.

2. Material and methods

2.1. Experimental diets

Of the five diets used in the feeding trials, three (A, B, and C) were semipurified diets formulated and prepared at Texas AgriLife Research Mariculture Laboratory (TARML) of the Texas A&M University System (Port Aransas, Texas, USA). Their compositions are given in Table 1. The digestible energy of the three semipurified diets was formulated at 3.54 ± 0.095 kcal/g. Passing through a Hobart A-200 extruder and being dried in an oven at 45 °C for 12 h, the diets were prepared by following the TARML protocol which had been described in a previous study (Gong et al., 2000). The other two were commercial diets containing 35 and 40% protein (Rangen, Buhl, Idaho, USA), included as reference diets 1 and 2 respectively. These two diets were included because they represent two of the most widely used shrimp feeds in the U.S. market for semi-extensive to semi-intensive (35/0, diet 1) and semi-intensive to intensive (40/5, diet 2) shrimp farming. Semipurified diet A, which outperformed the present commercial shrimp feeds from the U.S. market by 20% (Lawrence, unpublished data), was developed by TARML and has been widely used as a standard diet in many shrimp nutritional studies. Diets B and C were formulated by adjustment of the protein levels and sources used in diet A. In Table 2, the protein compositions and total lipids of the five diets are compared. The main difference between diets C and B was in crude protein level (35 and 20%, respectively) and soybean-meal content (16.7 and 0%, respectively), whereas diets A and C differed in the proportions of protein that was of marine origin (40 and 28.3%, respectively) and terrestrial plant origin, but total protein level was 35% in both (Table 2). All three semipurified diets were subjected to biochemical analysis for confirmation of their composition (Eurofins Scientific, Des Moines, Iowa, USA) following accredited methods by AOAC, AOCS, AACC, USDA and USP.

Table 1
Composition (percentage dry weight) of the three semi-purified experimental diets.

Ingredient	Diet A	Diet B	Diet C
Wheat starch[a]	29.60	46.42	30.37
Soybean protein isolated[b]	7.90	0.00	16.65
Fish meal, menhaden[c]	15.00	11.30	11.30
Squid muscle meal[b]	15.00	11.30	11.30
Menhaden fish oil[c]	0	0.80	0.80
Krill meal[b]	10.00	5.70	5.70
Stable vitamin C[b]	0.04	0.04	0.04
Diatomaceous earth[a]	2.00	1.77	2.20
Alginate[d]	2.00	2.00	2.00
Calcium carbonate[a]	2.00	1.61	2.08
Cellulose[e]	2.50	2.76	2.73
Cholesterol[f]	0.20	0.20	0.20
Potassium chloride[a]	2.00	2.17	2.13
Magnesium oxide[a]	1.50	1.55	1.54
Sodium chloride[a]	0.50	1.02	0.43
Soybean oil[a]	0.30	0.52	0.35
Phospholipids, 97%[f]	4.00	4.00	4.00
Sodium hexametaphosphate[e]	1.00	1.00	1.00
Calcium diphosphate[a]	3.00	4.38	3.72
Vitamin–mineral premix[b]	0.46	0.46	0.46

[a] MP Biomedicals, Solon, Ohio, USA.
[b] Zeigler Brothers, Gardners, Pennsylvania, USA.
[c] Omega Protein, Houston, Texas, USA.
[d] NutraSweet-Kelco Co., Chicago, Illinois, USA.
[e] Sigma-Aldrich Chemical, St. Louis, Missouri, USA.
[f] ADM, Decatur, Illinois, USA.

2.2. Shrimp

The two genetic lines of shrimp used in the study were designated as T-line and G-line. Both were SPF stocks, free of pathogens on the U.S. Marine Shrimp Farming Program working list, and met the criteria outlined in a review by Lightner (2005). Shrimp of the T-line, also known as the Industry Line, were purchased from the Oceanic Institute (OI, Waimanolo, Hawaii, USA) in 2007 at postlarval stage. The line was developed at the OI from a selection program initiated in 1995 under the U.S. Marine Shrimp Farming Program, and had been selected for TSV resistance for five generations initially and thereafter selected for both TSV resistance and rapid growth and achieved good performance in TSV resistance (Moss et al., 2011). The G-line was produced from shrimp broodstocks from a large joint research project, "Biosecure Zero-Exchange Shrimp Technology (BioZEST): A Paradigm Shift for the U.S. Industry," which was funded from 2001 through 2006 by the National Institute of Standards and Technology of the U.S. Department of Commerce. The BioZEST project was intended to develop an economically viable, environmentally responsible and sustainable system for the production of shrimp and included a genetic-selection program designed to breed shrimp suitable for

Table 2
Comparison of dietary crude protein and total lipid levels (%) and main protein sources (percentage dry weight) in three semipurified experimental diets and two commercial diets.

Diet	Total dietary protein (%)	Lipid (%)	Protein source of plant origin (%)	Protein sources of marine animal origin (%)
A	35	7.48	7.9	40.0
B	20	7.53	0	28.3
C	35	7.53	16.7	28.3
Ref. 1	35	8	NA	NA
Ref. 2	40	8	NA	NA

NA, information not available.